DEVELOPMENTAL BIOLOGY **200,** 82–91 (1998)
ARTICLE NO. DB988948

Intercellular Bridges between Epithelial Cells in the *Drosophila* Ovarian Follicle: A Possible Aid to Localized Signaling

Richard I. Woodruff*,[1] and Lewis G. Tilney†

**Department of Biology, West Chester University, West Chester, Pennsylvania 19383-8102; and*
†Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

In the epithelium of *Drosophila* ovarian follicles, cytoplasm-filled intercellular bridges connect epithelial cells. This study presents further descriptive information about the morphology of these intercellular bridges and the extent of their distribution. We also offer speculations concerning the possible developmental importance of the epithelial bridges. These bridges, whose luminal diameters averaged 0.25 μm, are smaller than those forming the ring canals joining germline cells: nor do they increase their size over time. The membranes limiting the bridges are lined on the cytoplasmic side with an electron-dense material to which is attached a monolayer of filaments which encircle the bridge. By decoration with the S1 fragment of myosin, these filaments are confirmed as actin filaments. Following disruption of gap junctional dye coupling by treatment with 1 mM octanol, microinjection of Lucifer yellow CH revealed the extent and distribution of follicle cell intercellular bridges to be confined to arrays of no more than eight cells/cluster, with many such independent clusters comprising the epithelium. Thus cell-to-cell movement throughout the epithelium of cytosolic regulatory molecules cannot occur via these intercellular bridges. However, weak signals affecting only one or a few cells in each cluster would be amplified throughout the group by spread through the intercellular bridges.

INTRODUCTION

Since the end of the 19th century, intercellular bridges (cytoplasmic bridges)[2] between cells have been known to occur (Platner, 1886; Fleming, 1891; Giardina, 1901). While easily observed between the nurse cells and oocytes of insect ovarian follicles (Platner, 1886), it was not until 1977 that epithelia surrounding the germline cells of some insects were also found to posses intercellular bridges. These were first visualized during ultrastructural studies of *Apis mellifica* (Ramamurty and Engles, 1977), *Aedes aegypti,* and *Stomoxys calcitrans* (Meola *et al.,* 1977), and the following year in *Culex pipiens* (Fiil, 1978) and *Drosophila* (Giorgi, 1978). From the time of discovery, it has not been known if the bridges interconnect all cells of the epithelium, or only clusters of a few cells each (Giorgi, 1978). While gap junctional communication in insects can pass molecules up to 3000 Da (Berdan, 1987; Bohrmann and Haas-Assenbaum, 1993), these channels can also narrow to a more restrictive size. Intercellular bridges, however, pass organelles such as ribosomes (Ramamurty and Engles, 1977; Giorgi, 1978). Thus the occurrence, frequency, and distribution of intercellular bridges in ovarian follicle epithelia have implications for the degree to which these cells may communicate with each other, and what size molecules and particles they may exchange.

We have performed ultrastructural studies which have visualized the bridges, and characterized the materials within them. The bridges average 0.25 μm in diameter, much smaller than the intercellular bridges (ring canals)

[1] To whom correspondence should be addressed. Fax: (610) 436-2183. E-mail, rwoodruff@wcupa.edu.

[2] Terms: The term ''egg chamber'' is often used to designate a *Drosophila* ovarian follicle. Across animal species, the term ''follicle'' is more generally used: here the terms are used interchangeably. The term ''follicle cell'' is used here to designate a cell of the epithelium of an ovarian follicle. In *Drosophila* the term ''ring canals'' has been used for the cytoplasmic connectives between cells. The terms ''intercellular bridges'' and ''cytoplasmic bridges'' are more generally used for such structures in all cell types and animals. To adhere to general usage and avoid confusion. in this paper we restrict ''ring canals'' to those intercellular bridges occurring between germline cells. While the term ''lumen'' implies a cavity, we have used it here to designate the largely cytosolic core of an intercellular bridge.

0012-1606/98 $25.00

connecting germline cells (Warn *et al.*, 1985; Tilney *et al.*, 1996). The bridges are lined with a peripheral region consisting of dense round particles and a more internal monolayer of filaments which encircle the lumen of the bridge. Decoration of filaments with subfragment 1 of myosin (S1) revealed them to be actin filaments. Ultrastructural details of bridge position and morphology are described below.

In *Drosophila* there are several cases of germline–somatic cell interactive signaling which control events in oogenesis (for review see Morgan and Mahowald, 1996). Among these events, perhaps the best known case is the cascade of events which controls establishment of the dorsoventral axis. This has been shown to depend on signals which pass between the oocyte and its follicular epithelium (Schupbach and Roth, 1994; Ray and Schupbach, 1996). In this example, a regional group of epithelial cells are stimulated through interaction with a specific area of the oocyte. In this and other examples (Schupbach and Roth, 1994; Morgan and Mahowald, 1996; Ray and Schupbach, 1996) many of the molecules involved are membrane bound. However, it is not unreasonable that some components of any such system spend time in solution. How could soluble control molecules remain localized? Particularly in light of the existence of intercellular bridges in dipteran epithelia, they might be able, over the several-day development of the follicle, to diffuse throughout the epithelium, negating the original regionalization.

To what extent cells of the epithelium are interconnected by bridges, and what are the implications of the bridges for cell–cell transfer of molecules involved in developmental control are questions first raised by Giorgi (1978). Intercellular bridges might link all epithelial cells, or bridges might link only those in small groups. To determine which of these conditions occurs in *Drosophila*, we used 1 mM octanol to disrupt gap-junctional dye coupling (Bohrmann and Haas-Assenbaum, 1993). Following loss of gap-junctional dye coupling, individual cells were injected with Lucifer yellow CH and the epithelium was checked for spread of dye, which could then only occur through intercellular bridges. The epithelia of stage 4 through stage 10 follicles were regularly found to be comprised of groups of cells, each group being interconnected by intercellular bridges, but with no bridges extending beyond the group. The number of interconnected cells in a group varied from two to eight cells, revealed by their ability to exchange dye, and the clearly defined edge of the fluorescent area.

MATERIALS AND METHODS

Culturing of Drosophila

Wild-type (Canton S) flies were obtained from Lynn Cooley (Yale University, New Haven, CT) and cultured using standard methods at 25°C. To obtain ovarioles with follicles at all stages in oogenesis we took newly emerged flies and placed them in new vials for 4 days. One day before dissection we added a small dollop of yeast paste to stimulate oogenesis. By the time of dissection the females had enormous abdomens with ovarioles swollen with egg chambers of all stages.

Drosophila Saline

One of the keys to adequate fixation was to dissect out the egg chambers in a *Drosophila* saline that was not hypertonic. To analyze adult *Drosophila* hemolymph, Singleton and Woodruff (1994) utilized a variation of freezing point depression which allowed analysis of submicroliter samples. By that method the tonicity of hemolymph from individual flies was found to average 255 mOsmol/liter, lower than that of any commonly used formulations of *Drosophila* saline. A physiological salt solution (PSS) that had the same major ion composition (Van der Meer and Jaffe, 1983) and osmolarity as *Drosophila* hemolymph was designed. We used this PSS made up of the following ingredients: 100 mM Na-glutamate, 25 mM KCl, 15 mM $MgCl_2$, 5 mM $CaSO_4$, 2 mM sodium phosphate buffer (pH 6.9). This solution is slightly hypotonic but it can be made isotonic by the addition of glucose if necessary.

Electron Microscope Procedures

For routine examinations, before or after detergent extraction egg chambers were fixed by immersion in a freshly made solution of 1% glutaraldehyde (from an 8% stock; Electron Microscope Sciences, Fort Washington, PA), and 1% OsO_4 in 0.05 M phosphate buffer (pH 6.2). Fixation was carried out for 45 min at 4°C. When the egg chambers were pipetted into fixative, small quantities of *Drosophila* saline were unavoidably introduced into the medium. Because the *Drosophila* saline interacts with the glutamate in the fixative, after 5 min the egg chambers were routinely put into fresh fixative.

After fixation the egg chambers were washed three times for 5 min each in 4°C water to remove excess phosphate and then stained *en bloc* overnight in 0.5% uranyl acetate. The specimens were then dehydrated in acetone and embedded in Epon. Thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined with a Philips 200 electron microscope. For this work, it was essential to examine specimens on uncoated grids.

Detergent Extraction

After dissection in PSS, egg chambers were extracted for 10 min at 4°C in a solution containing 1% Triton X-100, 3 mM $MgCl_2$, and 30 mM Tris (pH 7.5) with agitation on a rotating shaker. After 10 min, the detergent extracted egg chambers were fixed by immersion as described above. In some preparations we added phalloidin to the detergent solution and in the fixative to ensure that the filaments did not break down before fixation.

Decoration with Subfragment 1 of Myosin (S1)

After detergent extraction, some egg chambers were incubated in a solution containing 4 mg/ml S1 in 0.1 M phosphate buffer (pH 6.8) for 20 min at room temperature on a rotating table. The S1 had been previously prepared and kept frozen in small aliquots in liquid nitrogen at a concentration of 70 mg/ml (Tilney and Tilney, 1994). After decoration, specimens were washed in phosphate buffer and fixed at room temperature for 30 min in 1% glutaraldehyde in 0.1 M phosphate buffer (pH 6.8) containing 2% tannic acid. The

preparation was then washed in buffer and postfixed for 45 min at 4°C in 1% OsO_4 in 0.1 M phosphate buffer (pH 6.2) and processed further as above.

Rationale for Fixation Methods Used

To visualize the actin filaments one must extract from view as many soluble components as possible because, with conventional fixation, they obscure the actin cytoskeleton (see Tilney and Tilney, 1994). The key to fixation of the egg chambers was to use an isotonic or slightly hypotonic PSS so that the epithelial cells were not shrunken before fixation. We then used a fixative designed to extract the soluble proteins but maintain the cytoskeleton (see Tilney and Tilney, 1994). Briefly, if one uses osmium and glutaraldehyde together, the osmium pokes holes in the plasma membrane; these in turn allow small soluble proteins to escape, rather than being cross-linked in place by the fixative. The low pH of phosphate buffer in which the fixative was made tends to stabilized the actin filaments against the destructive properties of osmium. To further avoid degradation caused by the osmium medium, fixation was carried out at 4°C for no longer than 45 min (Tilney and Tilney, 1994). Even more details of the cytoskeleton could be visualized if the egg chamber was detergent extracted before fixation.

Lucifer Yellow Microinjections

Successful impalement of an individual epithelial cell required softening the hard acellular basement membrane. To accomplish this, following dissection follicles were incubated for 2–3 min in colagenase (Sigma) dissolved (1 mg/ml) in PSS. For most iontophoretic microinjections, they were then transferred to PSS containing 2 mM octanol dissolved in DMSO (final DMSO concentration 1%). Octanol in this concentration has been shown to disrupt gap to junctional dye coupling in *Drosophila* (Bohrmann and Haas-Assenbaum, 1993). The injection microelectrode was filled with a dilute solution of Lucifer yellow CH and placed on a stage-mounted Narashige MN-151 Emerson-type micromanipulator (Narishige Instruments, Japan). A current of approximately 50 nA was used to inject individual follicle epithelial cells.

Light Microscopy

An Olympus IMT-1 inverted microscope (Olympus Instruments, Japan) equipped with epi-illumination and the proper dichromatic mirrors, excitation filter, and barrier filters for observation of Lucifer yellow fluorescence was used for both brightfield and fluorescent light microscopy. Images were captured with a Dage-MTI 65 Newvicon video camera (Dage-MTI, Michigan City, IN). Fluorescent images were enhanced with an LKH-9000 unit (LK Hawke Inc., Research Triangle Park, NC) and recorded on a JVC BR-9000U 1/2 in. VHS time-lapse video recorder.

RESULTS

In our electron micrographs the intracellular bridges were always located at the level of the nucleus or near the apical end of the cell (Fig. 1). Although serial sections were not cut, we know that in a single section up to three bridges extend from a single cell, each one connected to a different

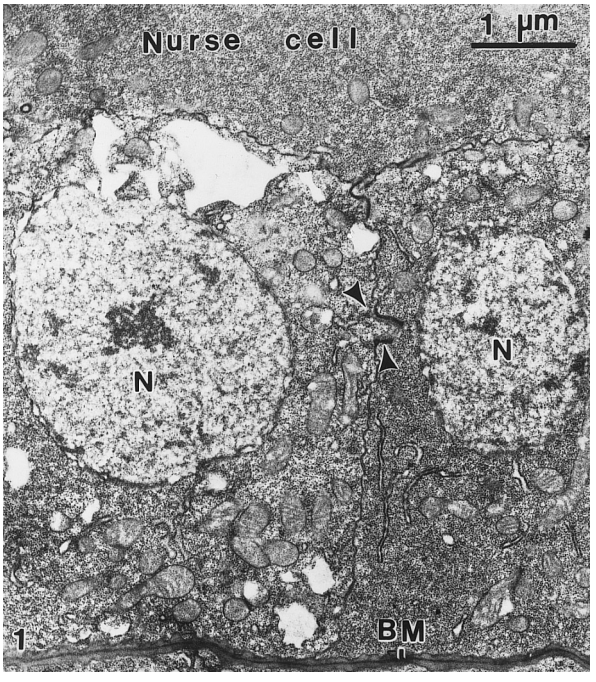

FIG. 1. Thin section cut through two adjacent epithelial cells in a stage 10 follicle. These cells can be identified by their prominent nuclei (N). Attached to their apical surfaces is a portion of an underlying nurse cell. Attached to their basal surface is the basement membrane (BM). Of interest to this report is the intercellular bridge (indicated by arrowheads) connecting the cytoplasm of these two epithelial cells. Bar, 1 μm.

adjacent follicle cell. We also found, in both detergent extracted and in unextracted tissue, several bridges that were tripartite, as if two bridges fused (Fig. 2). In these tripartite bridges, the thickness and morphology of the regions (described below) which line each bridge remained the same throughout each arm, giving evidence that they were stable structures and not the result of single bridges which had ruptured.

Each epithelial intercellular bridge, as seen in transverse or longitudinal sections, consists of a dense material, approximately 50 nm in thickness, attached to the cytoplasmic surface of the plasma membrane limiting the bridge. At higher resolution, and particularly in grazing sections through the intercellular bridges, this dense material could be seen to be made up of a series of small nearly spherical dense particles of unknown composition, each 50 nm in diameter, and attached side by side to form a nearly continuous layer. Inside this dense layer of particles was a monolayer of filaments which encircle the inside of the intercellular bridge like the wire surrounding a pasture (Figs. 3A, 3E, and 3F). Thus in a longitudinal section through a intercellular bridge they appeared as a linear row of dots each about 5 nm in diameter. In transverse section they appeared circular in profile (Figs. 3C and 3D). We have

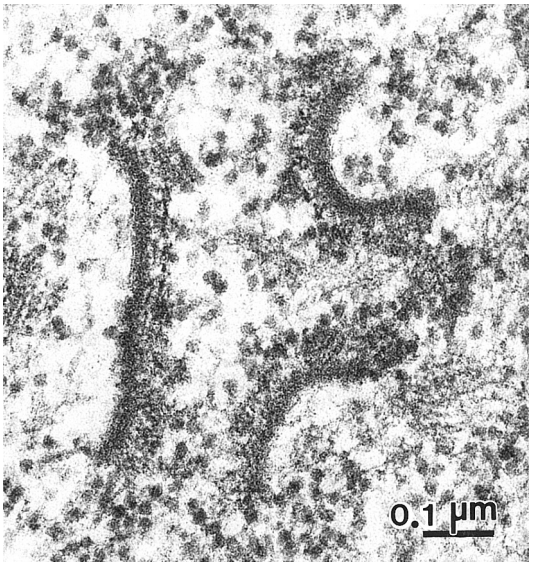

FIG. 2. Thin section through a tripartite bridge complex in detergent extracted follicle epithelial cells. Note that actin filaments encircle each of the channels. Bar, 0.1 μm.

been unable to determine if these filaments are like the hoops surrounding a whiskey keg, e.g., circular with no free ends, if these filaments are in a helical arrangement with a very low pitch, or if there are a number of short filaments.

We have measured the diameter of 35 follicular intercellular bridges (Table 1). The average diameter measured from the limiting plasma membrane on one side of the bridge to the plasma membrane on the other was 0.35 μm. The range of diameter was 0.30 to 0.41 μm. The true lumen of the bridge, however, must be located inside the filamentous layer. The lumen was 0.26 μm across in transverse sections and 0.23 μm in longitudinal section (Table 1) with a range of 0.16–0.28 μm.

The lengths of the bridges varied considerably, but not with follicle stage. When the cells were bound tightly to each other along their lateral surfaces, the intercellular bridge length could be less than 0.26 μm; yet in cells that were separated from each other, the intercellular bridges were as long as 0.90 μm. In all cases the walls of the intercellular bridges were decorated with the dense particles and the inner monolayer of filaments described above. Often elements of the rough surfaced ER were present in the intercellular bridge along with free ribosomes. In intercellular bridges that have presumably fused (so that in section they are tripartite) we found that each branch was limited by the dense layer of particles with its inner layer of filaments.

Prior to stage 6 we have found several follicular intercellular bridges that have within them microtubules. In favorable longitudinal thin sections, the microtubules end in the center of the intercellular bridge with the ends overlapping. These ends are surrounded by an electron dense material (Fig. 3B). Such images closely resemble the midbody of dividing cells. Thus these sections are consistent with earlier ideas that intercellular bridges are remnants of the midbodies that form during cytokinesis.

Detergent-Extracted Cells

When egg chambers were treated with the nonionic detergent Triton X-100, the membranes were totally solublized. This included, of course, the membranes of such organelles as mitochondria, ER, and yolk granules. The intercellular bridges could still be identified, however, by their dense peripheral coat and their monolayer of filaments. Of particular interest to this report is the interaction of these filaments with subfragments of myosin. When, after detergent extraction, the egg chambers were incubated with subfragment 1 of myosin, the filaments that line the intercellular bridge become thicker by the binding of S1 molecules. In neither cross nor longitudinal sections of normal thickness could ''arrowheads'' indicating the polarity of the filaments be clearly seen because the filaments are curved and it is hard to resolve individual arrowheads from the dense particle layer background immediately adjacent to the filaments. However, the fact that the filaments were 50 Å thick prior to decoration (Figs. 3C and 3D) and two to three times thicker after decoration with S1 (Figs. 3G and 3H) is good evidence that these filaments are actin filaments.

Lucifer Yellow Injections

To determine the extent to which an epithelium was interconnected by these intercellular bridges, Lucifer yellow CH was microinjected into individual cells. Figure 4B shows an example of the dye spread which occurred when Lucifer yellow was injected into epithelial cells of control follicles incubated in PSS only (no octanol). In addition to any intercellular bridges present, all epithelial cells were interconnected by gap junctions, and each also had gap junctional communication with the oocyte. Through the gap junctions the dye was able to spread ever increasing distances through the epithelium, as evidenced by the lack of a sharply defined edge to the fluorescence. Dye could also be seen in the oocyte, demonstrating the existence of open gap-junctional communication between the two cell types. Furthermore, from the injected cell dye spread outward evenly in all directions so that the injected cell was always at the center of an expanding patch of fluorescent cells.

In another set of follicles, gap-junctional dye coupling was disrupted by treatment with 1 mM octanol (Bohrmann and Haas-Assenbaum, 1993). Following this treatment, the only way for dye to move from one cell to another was through intercellular bridges, confirmed by the subsequent failure of dye to pass from any epithelial cell to its oocyte. Dye spread following injection of individual cells was monitored, revealing the number of cells bridged in a

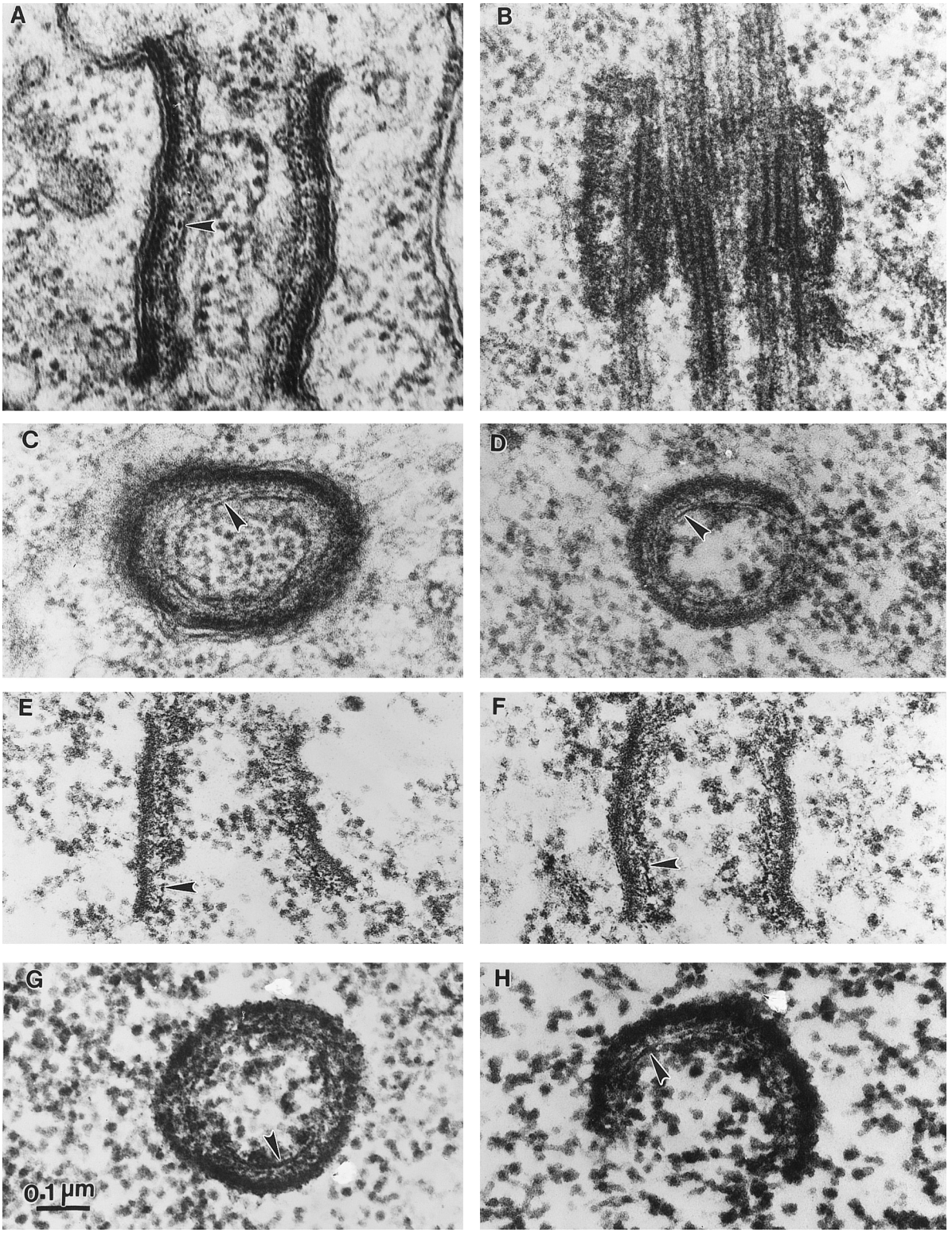

FIG. 3. All micrographs in this plate are at the same magnification. Bar, 0.1 μm. (A) Longitudinal section cut through an intercellular bridge between two follicle cells. Inside the plasma membrane of the two cells is a dense material and within that are tiny dots which are the actin filaments cut in transverse section (<). In the lumen of the bridge are larger dots which are ribosomes. (B) Longitudinal section

TABLE 1
Dimensions in μM ($\pm$SEM) of Epithelial Cell Intercellular Bridges

	Diameter	Range	Lumen diameter	Range	Length	Range
Transverse sections	0.34 ± 0.02 (n = 9)	0.30–0.39	0.26 ± 0.01 (n = 9)	0.19–0.28	0.40 ± 0.02 (n = 9)	
Longitudinal sections	0.37 ± 0.03 (n = 25)	0.30–0.41	0.23 ± ? (n = 26)	0.16–0.28	0.46 ± ? (n = 26)	0.26–0.90

cluster as well as any pattern they formed. Figures 4C and 4E show typical microinjections into octanol treated follicles, as seen in side view. Note the sharp edge to the regions of fluorescent cells, and the lack of any fluorescence in the underlying oocytes. In Fig. 4D, the location of the microinjection needle can be seen in the brightfield image, while in the fluorescence image its position may be determined by the small indentation it made. Note that the dye has not spread equally in all directions from the injection site. In this example, the microinjected cell was located just one cell away from the border of an interbridged cluster. Following microinjection, each follicle was carefully rolled on its long axis so that the injected cell was directly centered over the objective of the inverted microscope. In this way the number and pattern of bridged cells could be more easily seen. In Fig. 5A, a group of eight interbridged cells from a stage 10 follicle can be seen in surface view. The cells form a curved line, nearly describing a circle. The inset diagram numbers the cells in order of the intensity of their fluorescence, and shows the prior location of the now absent injection needle. Patterns were often linear (Fig. 5B), but sometimes appeared as roughly symmetrical rounded patches (Fig. 5C). No regularly occurring specific patterns were observed. Since intercellular bridges linked cells in groups of no more than eight, it was possible to survey several regions of a single follicle, as well as various regions of separate follicles. Throughout the epithelium over the oocyte, including regions adjacent to the oocyte nucleus after stage 8, the same results were obtained. Margolis and Spradling (1995) have used immunolabeling to follow cell-line clones in the epithelium of *Drosophila* follicles. At developmental stage 10, their micrographs show both linear arrays and ''rounded'' patches morphologically similar to those shown here .

Of 70 successful microinjections, 8 were into cells which showed no dye transfer to any neighbors nor to the oocyte. These are assumed to be solitary cells which lacked any intercellular bridges. The results of the remaining 62 microinjections can be seen in Tables 2 and 3.

DISCUSSION

The Ultrastructure of Epithelial Intercellular Bridges

The follicle cells form a single epithelial layer around oocyte and nurse cells, with the apical end of each cell adjacent to the germline cells and the basal end adjacent to the basement membrane forming the outer surface of the egg chamber. Earlier studies (Cummings *et al.*, 1969; Giorgi, 1978) have suggested that there are approximately 1200 follicle cells surrounding the *Drosophila* egg chamber at stage 6, and that this number does not subsequently change. These cells were derived from division of approximately 80 cells present as the follicle leaves the germarium (stage 1) (King and Vanouceck, 1960; King *et al.*, 1968; King, 1970). This requires, on average, four divisions (1200/80 = 15) of each cell. From the work of Giorgi (1978) we know that some of the follicle cells are connected by intercellular bridges. In our study, these average 0.35 μm in diameter,

through a detergent extracted intercellular bridge that formerly connected two follicle cells. Of interest is that within the bridge proper, encircled by the dense material and its associated filaments, are microtubules. These microtubules in the center of the ring canal comprise the midbody of this stage 6 follicle. (C) Transverse section of an intercellular bridge connecting two follicle cells (not detergent extracted). Within the plasma membrane is some dense material. Running around the inside of this are filaments (arrowhead (D) Transverse section through a detergent extracted intercellular bridge that formerly connected the cytoplasm of two follicle cells. Of interest are the filaments that encircle the bridge (arrowhead) (E and F) Thin longitudinal sections through two detergent extracted intercellular bridges that formerly connected adjacent follicle cells. The intercellular bridge is lined by some dense material within which is a monolayer of tiny dots (arrowhead). These dots are the actin filaments cut in transverse section. (G and H) The follicular epithelium was detergent extracted then incubated in S1 subfragment of myosin before fixation. In these two transverse thin sections one can distinguish filaments that are two to three times thicker than filaments depicted in C and D. At certain positions (indicated by arrowhead) one can see the arrowhead configuration of S1 decorated filaments.

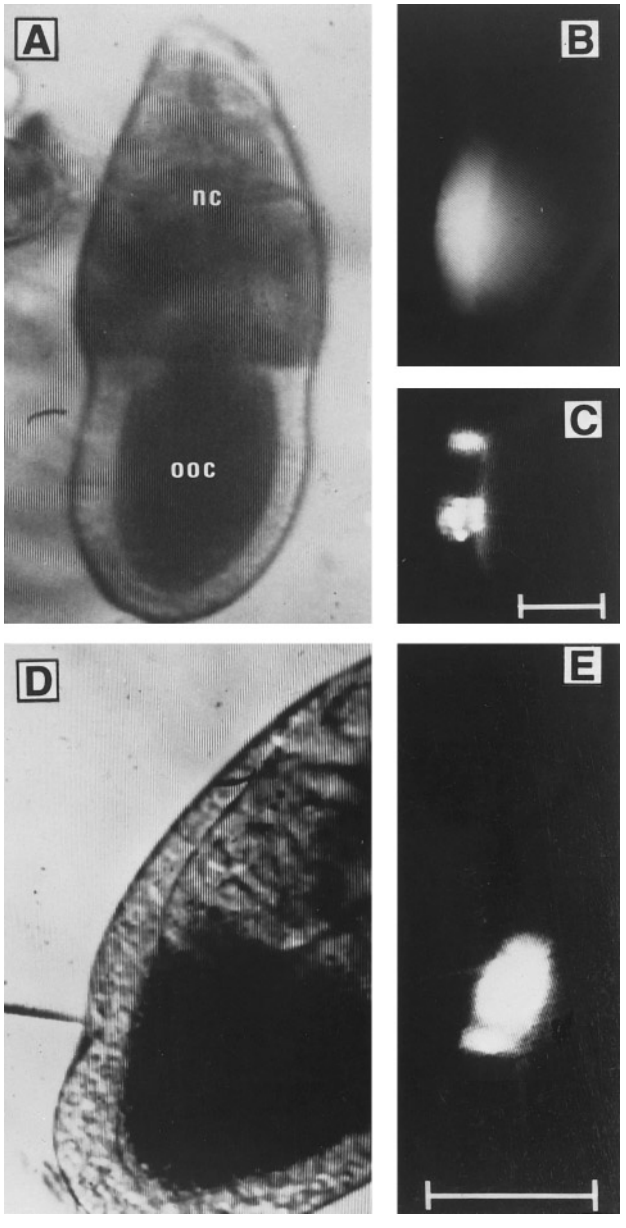

FIG. 4. Brightfield and fluorescence micrographs of Lucifer yellow microinjected into individual follicle cells of stage 9 and 10 *Drosophila* follicles. (A) An example of a stage 10 follicle (B) When injected into stage 10 control epithelia (not treated with octanol) the dye spreads evenly and continually in all directions through the epithelium and into the oocyte (5 min postinjection). (C) Following octanol treatment dye coupling via gap junctions is disrupted, leaving intercellular bridges the only way for dye to move. (20 min postinjection, upper injection into a solitary cell, lower injection into a cluster of eight cells. Note the sharp edges to the fluorescent areas and the lack of dye in the oocyte.) D and E show injection into the epithelium of a stage 9 follicle treated with octanol, 20 min postinjection. The injection needle is visible in the brightfield view, in the fluorescence view its position is revealed by the slight indentation. ooc, oocyte; nc, nurse cells. Scale bar for A, B, and C, 50 μm. Scale bar for D and E, 50 μm.

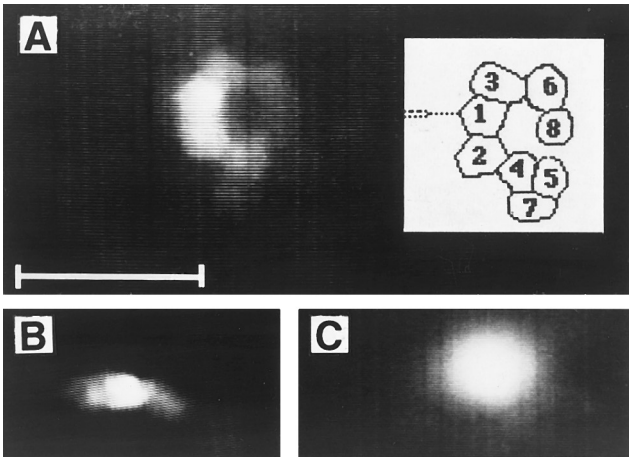

FIG. 5. Fluorescence micrographs of epithelia from octanol treated stage 10 follicles, surface view, 20 min postinjection. (A) Linear array of eight cells in the form of a nearly closed circle. The inset diagram shows the former position of the now absent injection needle, and numbers the cells in order of their relative fluorescence. (B) Linear array of interbridged cells. (C) Eight cells interconnected by intercellular bridges and clustered in a roughly circular patch. Scale bar, 50 μm.

much smaller than the intercellular bridges (ring canals) connecting adjacent nurse cells (0.5 to 10 μm in diameter) (Warn *et al.*, 1985; Tilney *et al.*, 1996).

While it is tempting to assume the presence of actin filaments in a structure, formation of which has included a cytokinetic contractal ring, the issue must always remain in doubt until proven. A case in point is represented by the intercellular bridges formed among germ line cells in male *Drosophila.* Hime *et al.* (1996) have shown there to be no actin filaments detectable in these stable bridges, at least at the level of detection for the method employed. In the present study, decoration with S1 confirms the presence of actin filaments within the bridges joining follicle epithelial cells. In nurse cells the ring canals generated in the germarium have a lumen which increases in size 20-fold from 0.5 μm in diameter (stage 1) to 10 μm in stage 10 egg chambers

TABLE 2

Frequency of Cluster Size in Sample Population (62 Interbridged Cell Clusters)

Number cells/ bridged cluster	Number of occurrences in population sampled	% Population sampled
8	19	30
6	11	17.7
4	25	40.3
2	7	11.3

TABLE 3
Average Number of Bridged Cells in a Cluster by Developmental Stage and Overall

Developmental stage	Avg. *n* cells/cluster (±SD)	*n*
4–7	3.6 ± 1.5	6
8	4.9 ± 2.6	11
9	4.9 ± 1.8	24
10	6.6 ± 1.7	21
All stages combined	5.4 ± 2.1	62

(Warn *et al.*, 1985; Tilney *et al.*, 1996). Connected to this increase in diameter is an increase in the amount of F actin so that by stage 10, if the filaments were attached end to end, each ring canal would have ≈1 inch (23 mm) of actin filaments. In contrast were the diameters of the intercellular bridges of follicle cells, which remained unchanged at only 0.35 μm throughout oogenesis. Likewise the amount of actin filaments in each bridge also remained unchanged. Unlike the ring canals among nurse cell, where bundles of actin are seen, the filaments in the follicular intercellular bridges appear as a monolayer attached to the dense material lining the membrane.

What controls the number and organization of the actin filaments in intercellular bridges of these two systems is clearly different, but the basic structure remains the same. We presume that the actin filaments in both cases give rigidity to this bridge because if actin filaments do not appear in the ring canals, such as in the mutant *hts* (Yue and Spradling, 1992), the ring canals collapse and nurse cell to oocyte transport is terminated, leading to sterility.

The Extent and Pattern of Epithelial Intercellular Bridges

In the epithelium surrounding *Drosophila* germline cells, interbridged cell clusters consisting of four or eight cells predominated, collectively constituting 70% of the population sampled. This is what one would expect if all cells in a cluster divide until a fixed number is reached, and this certainly is the most likely scheme where the interbridged cells formed a rounded patch (Fig. 5C). In clusters of this type, one would expect to find some cells with several bridges, and some with only one. Indeed, in *Apis* such a distribution of bridges within a cluster seems to occur (Ramamurty and Engles, 1977). This type of division pattern would also be consistent with our ultrastructural evidence for *Drosophila* in which cells with tripartite bridges and cells with three separate bridges were encountered. In clusters of eight cells produced in this manner, one would expect there to be two cells with three bridges, two cells with two bridges, and four cells with but a single bridge each. However, there were also many ''clusters'' in which the cells appeared in a linear array (Figs. 5A and 5B), and in these there must be only two bridges per cell. The variability in number and pattern suggests that incomplete cytokinesis and formation of stable intercellular bridges is not as tightly regulated in the epithelium as in the germline.

The significance of intercellular bridges between follicle epithelial cells derives from recent discoveries concerning maternal effect genes and the regulation of early development in *Drosophila*. The establishment of both the embryonic anterior–posterior and dorsoventral axes includes interactive signaling between the oocyte and the epithelium which surrounds it (for reviews, see both Schupbach and Roth, 1994, and Ray and Schupbach, 1996). Many of the identified regulatory molecules are membrane-bound (Schupbach and Roth, 1994) and are thus contained within not only a particular epithelial region, but within individual cells. However, the downstream results of the interactions include stimulation of several genes and utilization of their products, and it is reasonable to assume that some of the elements in these event cascades must exist free within the cytosol.

Systems such as those mentioned above depend upon containing the signal within a localized area (Neuman-Silberberg and Schupbach, 1994; Roth *et al.*, 1995; Neuman-Silberberg and Schupbach, 1996), yet there are two routes by which cytosolic components might spread. One route is through the gap junctions which normally link all cells within a follicle, but which have a molecular size restriction of <3000 Da (Berdan, 1987; Bohrmann and Haas-Assenbaum, 1993), smaller than most regulatory molecules in such systems. The second possible route would be through the much larger intercellular bridges. If all cells in the epithelium were interconnected by intercellular bridges, a mechanism would be needed to prevent the diffusion of cytosolic elements of any control cascade, and the loss of the regionalization upon which they are assumed to depend. This is particularly true when the diffusion is through epithelial cells and might occur over days rather than within the cytoplasm of the early precellularized embryo; a period which lasts only hours. Evidence presented here demonstrates that within the epithelium of the *Drosophila* ovarian follicle, cell–cell communication via open intercellular bridges of cytoplasm is limited to individual clusters of at most eight cells.

Speculations on a Possible Role for the Epithelial Intercellular Bridges

In the germarium each cluster of germline cells is surrounded by profollicle cells which become the follicle epithelium. In germarium regions 2 and 3, mitotic cell divisions increase the number of epithelial cells (Spradling, 1993). Intercellular bridges in this region were noted by Giorgi (1978), who described them as being ''of unusual morphology,'' and containing microtubules running parallel to the long axis of the bridge. Based on evidence presented here, these may represent spindle remnants and cleavage furrows which eventually com-

plete division. By stage 1, as they leave the germarium, the epithelium consists of 80 cells/follicle (King and Vanouceck, 1960; Giorgi, 1978). If cell divisions forming persistent intercellular bridges had occurred while the follicle was still in the germarium, cell clusters would consist of more than 16 cells each. Alternatively, bridges could have been formed prior to stage 1, and the additional cells found in the epithelia of later stages could be derived from solitary cells which always complete cytokinesis. If solitary cells accounted for all of the additional cells formed after stage 1, they would be the most numerous in the population. However, in 70 microinjections, only 8 were into solitary cells. Instead the change in mode of cytokinesis must have occurred later, when there were approximately 160 cells making up the epithelium. This means that the epithelium must have, at a time later than stage 1, changed from a mode of cytokinesis which eventually completes the separation of sibling cells to a mode which results in persistent intercellular bridges. Could there be a possible adaptive advantage to such a change in this basic cell process? Since, to avoid spreading a regionalized signal, the number of cells in an interbridged cluster must be small, why retain intercellular bridges at all? We speculate that an evolutionary advantage may be to smooth and amplify a signal received by follicle cells. In the example of ''dorsalization,'' increase in the strength of the Gurken signal has been shown to result in greater numbers of follicle cells being stimulated over a wider area of the epithelium (Neuman-Silberberg and Schupbach, 1994), yet insufficient amounts of Gurken fail to establish dorsalization. The dilemma in which a strong signal spreads too far, but a weak one fails to stimulate sufficient number of local cells may be solved by the pattern of intercellular bridges within the epithelium. With epithelial cells interbridged in clusters of 8 cells each, theoretically local signal concentration need only be strong enough to stimulate 1 of every 8 cells encountered. Since all cells in each 8-cell cluster are interbridged, even if only 1 cell of the cluster were to be stimulated all would equally share downstream molecules from the one responding cell. Thus the signal would be both amplified and smoothed throughout the target area, while in more peripheral regions it would remain below sufficient strength for stimulation.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health GM-32909 (R. I. Woodruff) and GM-52857 (L. G. Tilney).

REFERENCES

Berdan, R. C. (1987). Intercellular communication in arthropods: biophysical, ultrastructural, and biochemical approaches. *In* ''Cell-to-Cell Communication'' (W. C. De Mello, Ed.), pp. 299–370. Plenum, New York.

Bohrmann, J., and Haas-Assenbaum, A. (1993). Gap junctions in ovarian follicles of *Drosophila melanogaster*: inhibition and promotion of dye-coupling between oocytes and follicle cells. *Cell Tissue Res* **273,** 163–173.

Cummings, M. R., Brown, N. M., and King, R. C. (1969). The cytology of the vitellogenic stages of oogenesis in *Drosophila melanogaster*. I. General staging characteristics. *J. Morphol.* **128,** 427–442.

Fiil, A. (1978). Follicle cell bridges in the mosquito ovary: Syncytia formation and bridge morphology. *J. Cell Sci.* **31,** 137–143.

Fleming, W. (1891). Neue beitrage zur kenntniss der zelle. *Arch. Mikrosk. Anat.* **37,** 685–751.

Giardina, A. (1901). Origine dell' oocite e delle cellule nutrici nei *Dytiscus. In. Mschar. Anat. Physiol.* **18,** 417–484.

Giorgi, F. (1978). Intercellular bridges in ovarian follicles of *Drosophila melanogaster. Cell Tissue Res.* **186,** 413–422.

Hime, G. R., Brill, J. A., and Fuller, M. T. (1996). Assembly of ring canals in the male germ line from structural components of the contractile ring. *J. Cell Sci.* **109,** 2779–2788.

King, R. C. (1970). ''Ovarian Development in *Drosophila melanogaster,* Academic Press, New York.

King, R. C., Aggarwal, S. K.. and Aggarwal, U. (1968). The development of the female *Drosophila* reproductive system. *J. Morphol.* **124,** 143–166.

King, R. C., and Vanouceck, E. G. (1960). Oogenesis in adult *Drosophila milanogaster.* X. Studies on the behavior of the follicle cells. *Growth 24,* 333–338.

Margolis, J., and Spradling, A. (1995). Identification and behavior of epithelial stem cells in the *Drosophila* ovary. *Development* **121,** 3797–3807.

Meola, S. M., Mollenhauer, H. H., and Thompson, J. m. (1977). Cytoplasmic bridges within the follicular epithelium of the ovarioles of two diptera, *Aedes aegypti* and *Stomoxys calcitrans. J. Morphol.* **153,** 81–85.

Morgan, M. M., and Mahowald, A. P. (1996). Multiple signaling pathways establish both the individuation and the polarity of the oocyte follicle in *Drosophila. Arch. Insect Biochem. Physiol.* **33,** 211–230.

Neuman-Silberberg, F. S., and Schupbach, T. (1994). Dorsoventral axis formation of *Drosophila* depends on the correct dosage of the gene gurken. *Development* **120,** 2457–2463.

Neuman-Silberberg, F. S., and Schupbach, T. (1996). The *Drosophila* TGF-α-like protein Gurken: Expression and cellular localizaton during *Drosophila* oogenesis. *Mech. Dev.* **59,** 105–113.

Platner, G. (1886). Die karyokinese bie den Lepidopteren als Grundlage fur eine theorie der zellteilung. *Int. Mschr. Anat. Physiol.* 341–398.

Ramamurty, P. S., and Engles, W. (1977). Occurrence of intercellular bridges between follicle epithelial cells in the ovary of *Apis mellifica* queens. *J. Cell Sci.* **24,** 195–202.

Ray, R. P., and Schupbach, T. (1996). Intercellular signaling and the polarization of body axes during *Drosophila* oogenesis. *Genes Dev.* **10,** 1711–1723.

Roth, S., Neuman-Silberberg, F. S., Barcelo, G., and Schupbach, T. (1995). *cornichon* and the EGF receptor signaling process are necessary for both anterior–posterior and dorsal–ventral pattern formation in *Drosophila. Cell* **81,** 976–978.

Schupbach, T., and Roth, S. (1994). Dorsoventral patterning in *Drosophila* oogenesis. *Curr. Opin. Genet. Dev.* **4,** 502–507.

Singleton, K., and Woodruff, R. I. (1994). The osmolarity of adult *Drosophila* hemolymph and its effect on oocyte–nurse cell electrical polarity. *Dev. Biol.* **161,** 154–167.

Spradling, A. C. (1993). Development genetics of oogenesis. *In* ''The Development of *Drosophila melanogaster*'' (M. Bate and A. Martinez Arias, Eds.), pp. 1d–70. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Tilney, L. G., and Tilney, M. S. (1994). Methods to visualize actin polymerization associated with bacterial invasion. *Methods. Enzymol.* **236,** 476–481.

Tilney, L. G., Tilney, M. S., and Guild, G. (1996). Formation of actin filament bundles in the ring canals of developing *Drosophila* follicles. *J. Cell Biol.* **133,** 61–74.

Van der Meer, J. M. and Jaffe, L. F. (1983). Elemental composition of the perivitelline fluid in early *Drosophila* embryos. *Dev. Biol.* **95,** 249–252.

Warn, R. M., Gutzeit, H. O., Smith, L., and Warn, A. (1985). F-actin rings are associated with the ring canals of the *Drosophila* egg chamber. *Exp. Cell Res.* **157,** 355–363.

Yue, L., and Spradling, A. C. (1992). Hu-li tai shao, a gene required for ring canal formation during *Drosophila* oogenesis, encodes a homolog of adducin. *Genes Dev.* **6,** 2443–2454.

Received for publication January 15, 1998
Revised March 30, 1998
Accepted April 21, 1998